

Review

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Review

Virus Meets Chemistry: Chemical, Genomic, and Structural Strategies for Targeted Bacteriophage Therapeutics

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Abstract

Objectives: To explore the molecular chemistry and structural biology of bacteriophages as precision-guided therapeutic agents. This review reframes phages as programmable nanomachines governed by defined chemical interactions, focusing on their relevance to antimicrobial resistance, targeted lysis, and synthetic modification. **Materials and Methods:** A systematic review was conducted using recent peer-reviewed literature from PubMed, Scopus, and Google Scholar, covering bacteriophage structural biology, enzymatic lysis mechanisms, chemical modifications, genomic annotation, and bioengineering techniques. Studies were selected based on relevance to molecular chemistry, nanotechnology, and therapeutic development. **Results:** Bacteriophages demonstrate ligand-specific host recognition, capsid-mediated genome packaging, and enzymatic lysis using holins and endolysins. Advances in PEGylation, surface conjugation, and CRISPR engineering have expanded their therapeutic potential. Genomic tools now enable personalized phage matching, while hybrid phage-nanoparticle systems enhance targeting and delivery. **Conclusions:** Phages can be rationally designed as chemically programmable antimicrobials. Their structure–function relationship, enzymatic precision, and genomic adaptability position them as promising agents in the fight against multidrug-resistant pathogens. Integration of chemistry, bioinformatics, and synthetic biology enables development of next-generation phage therapeutics.

Keywords: Bacteriophages; phage therapy; molecular chemistry; synthetic biology; endolysins; holins; nanomedicine; surface modification; genomics; bioinformatics

Introduction

Antibiotic resistance remains a global health threat, with multidrug-resistant (MDR) infections rendering conventional therapies ineffective. In this context, bacteriophages (phages) viruses that infect and lyse bacteria have re-emerged as promising alternatives due to their species-specificity, self-amplification, and microbiome preservation [1,2].

Structurally, phages are macromolecular assemblies that function like supramolecular nanomachines. Their infection cycle adsorption, DNA injection, replication, and lysis is governed by precise molecular interactions [3]. Unlike broad-spectrum antibiotics, phages recognize specific bacterial receptors through evolved tail fibers and baseplate proteins. These interactions mirror ligand-receptor binding in molecular chemistry [4].

Key to their antibacterial action is the lytic phase, where enzymes such as **endolysins** cleave peptidoglycan bonds, while **holins** create timed membrane pores that release progeny [5]. These enzymes exhibit bond-specific catalysis, often relying on conserved catalytic residues or metal ions for function [6]. Their specificity reduces off-target effects and supports narrow-spectrum therapeutic use.

Recent advances in **surface chemistry** like PEGylation and ligand conjugation—have enhanced phage pharmacokinetics and targeting ability [7]. In parallel, genomic sequencing and machine learning now enable rapid phage annotation, host prediction, and personalized therapeutic development [8,9].

By integrating chemical biology, synthetic engineering, and bioinformatics, phages can now be reframed as programmable nanomachines designed for precision medicine. This review outlines the structural, chemical, and genomic mechanisms that underpin this transformation.

Molecular Architecture of Bacteriophages

Bacteriophages, particularly tailed types within the order *Caudoviricetes*, exhibit a conserved modular structure comprising a protein capsid, a tail for genome delivery, and tail fibers or baseplates for host recognition [10]. These structures are stabilized by non-covalent interactions such as hydrogen bonds, electrostatic forces, and hydrophobic packing mirroring principles of supramolecular chemistry [11].

The **capsid** is typically icosahedral and encloses tightly packed double-stranded DNA. Internal pressures from DNA compaction (~50 atm) drive injection upon infection [12]. This pressure is stabilized by divalent cations and DNA–protein interactions that protect the genome during extracellular transport [13].

The **tail structure** varies by phage family: *Myoviridae* exhibit contractile tails, *Siphoviridae* have flexible tails, and *Podoviridae* possess short stubs [10]. Tail fibers interact with specific bacterial receptors, including lipopolysaccharides, outer membrane proteins, or teichoic acids. These ligand–receptor interactions are chemically selective and determine host range [14]. **Figure 1** presents a schematic representation of a canonical tailed bacteriophage, highlighting its icosahedral capsid encasing double-stranded DNA, a contractile tail sheath, baseplate, and terminal tail fibers. The tail fibers are depicted engaging specific bacterial surface receptors such as lipopolysaccharides or outer membrane proteins demonstrating molecular selectivity akin to ligand–receptor binding interactions in synthetic chemistry.

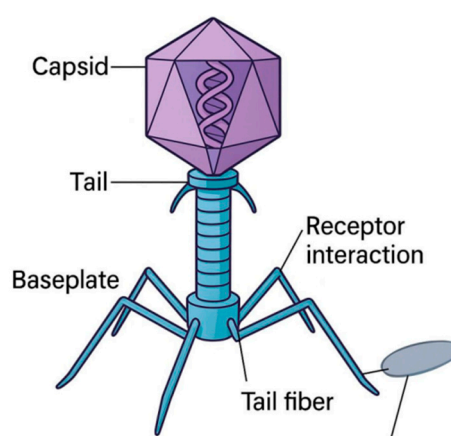


Figure 1. Structural schematic of a tailed bacteriophage showing capsid, contractile tail, baseplate, and tail fibers binding to host receptors.

Phage self-assembly is a chemically guided process involving scaffolding proteins, chaperones, and energy-dependent DNA-packaging motors. The resulting architecture functions as a targeted nanodevice engineered by evolution for delivery precision and stability in harsh conditions [15].

Understanding this architecture supports rational design of phages with enhanced infectivity, structural stability, or engineered surfaces for therapeutic use.

Mechanistic Chemistry of Phage–Bacteria Interaction

Phage–bacteria interaction is a multi-stage process governed by precise chemical recognition and structural transitions. Each step adsorption, DNA injection, replication, and lysis demonstrates fine molecular coordination [16]. Illustrated in **Figure 2**, stepwise chemical and structural processes of phage infection include: (1) host recognition via tail fiber ligand binding; (2) genome injection; (3) replication; (4) enzymatic lysis via holins and endolysins; and (5) phage release. Each stage emphasizes molecular interactions and catalysis.

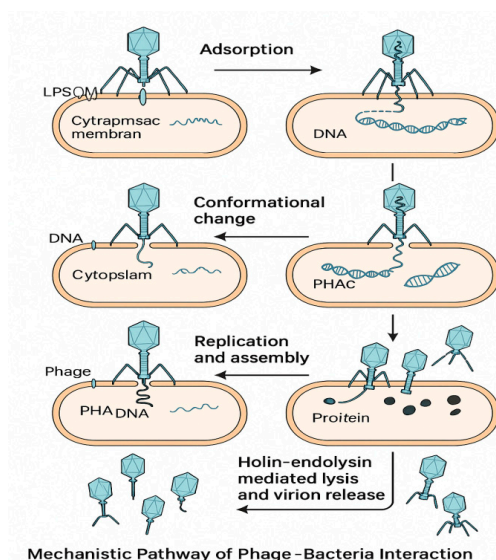


Figure 2. Life cycle of a lytic bacteriophage, illustrating attachment, DNA injection, replication, and enzymatic lysis.

Adsorption begins with reversible binding between phage tail fibers or receptor-binding proteins (RBPs) and bacterial surface molecules such as lipopolysaccharides (LPS) or outer membrane proteins. These interactions rely on hydrogen bonding, hydrophobic effects, and electrostatic complementarity, mimicking host–ligand specificity in synthetic biology [17].

Upon irreversible attachment, **conformational changes** in the tail structure trigger genome injection. In *Myoviridae*, tail sheath contraction propels a hollow tube into the bacterial cell wall. This process is powered by the stored pressure within the capsid and completed in milliseconds [18].

Following replication, phages initiate **lysis** through enzymes that degrade the host envelope. Holins form pores in the cytoplasmic membrane, while endolysins hydrolyze glycosidic or amide bonds in the peptidoglycan. These enzymes exhibit site-specific catalysis, minimizing off-target effects and microbial resistance development [19].

The chemical choreography of these steps reveals phages as self-contained, time-regulated molecular machines efficient, selective, and programmable for targeted antimicrobial action [20].

Enzymatic Lysis: Endolysins, Holins, and Catalytic Strategies

Endolysins and holins orchestrate the final step of phage infection host cell lysis. Their enzymatic mechanisms are chemically selective, allowing phages to kill bacteria with surgical precision [21]. As illustrated in **Figures 3A–3E**, endolysins can be classified into at least five distinct enzymatic classes based on their cleavage sites and catalytic modes.

1. **Endolysins** are peptidoglycan hydrolases that cleave bonds within the bacterial cell wall. They are categorized by their catalytic targets:
 - (a) **N-acetylmuramidases**: cleave β -1,4 bonds between MurNAc and GlcNAc.
 - (b) **Amidases**: hydrolyze amide bonds between MurNAc and L-Ala.

- (c) **Endopeptidases:** target peptide bridges.
- (d) **Transglycosylases:** generate 1,6-anhydro rings [22].

These enzymes often function via acid-base catalysis, utilizing conserved glutamic/aspartic residues, and sometimes metal cofactors (e.g., Zn^{2+}) for transition state stabilization [23].

2. Holins are small, membrane-integrating proteins that form pores at a precisely timed phase during phage replication. Their oligomerization enables endolysins to access the peptidoglycan. In Gram-negative hosts, additional proteins like spanins assist in disrupting the outer membrane [24].

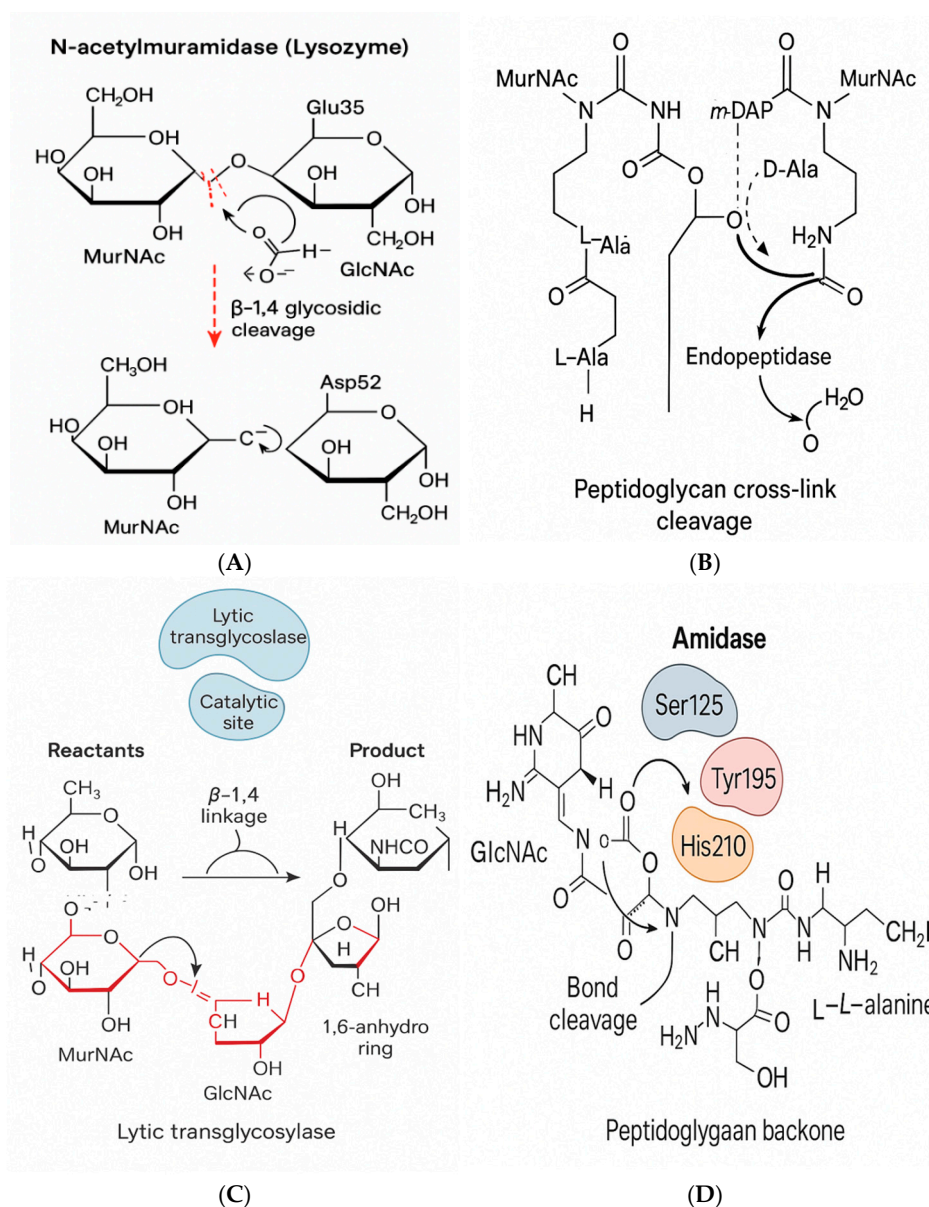
These lytic proteins are being engineered into:

1. **Enzybiotics:** recombinant antimicrobials with high specificity.
2. **Fusion constructs:** endolysins linked to targeting peptides.
3. **Synthetic holins:** mimicked by amphipathic drug delivery peptides [25].

Together, endolysins and holins form a chemically elegant, bond-specific, and temporally regulated antibacterial strategy.

Phage Engineering and Surface Chemistry

Modern chemistry and bioengineering have enabled the reprogramming of phages into customizable delivery platforms.



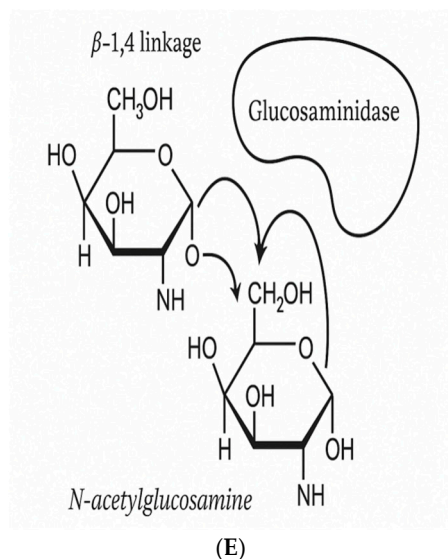


Figure 3. Catalytic mechanisms of phage-derived enzymes. (A) Muramidase cleaving β -1,4 glycosidic bonds between GlcNAc and MurNAc. (B) Endopeptidase breaking peptide cross-links in peptidoglycan. (C) Transglycosylase forming 1,6-anhydro rings via cyclization.

Capsid Engineering

Using click chemistry (e.g., CuAAC, SPAAC), amide coupling, or thiol–maleimide conjugation, researchers have functionalized capsid and tail proteins with targeting ligands, imaging agents, or stimuli-responsive moieties [26].

PEGylation

PEGylation enhances pharmacokinetics by extending circulation time, reducing immune clearance, and improving tissue diffusion, all while retaining infectivity [27].

Phage Display

By fusing peptides or proteins to capsid coat proteins (typically pIII or pVIII), phage display allows the identification and presentation of ligands targeting specific bacterial strains or tumor cells [28].

Phage–Nanoparticle Hybrids

Hybrid constructs merge phages with synthetic materials:

- **Gold–phage:** For photo-thermal ablation of biofilms.
- **Magnetic–phage:** For guided drug delivery.
- **Drug–phage:** Co-deliver antibiotics and matrix-degrading enzymes [29].
- **Synthetic Phages**

De-novo synthesis of phage genomes and structural proteins has enabled the design of synthetic phages with enhanced host specificity, reduced immunogenicity, and customized payloads [30].

These developments showcase phages as chemically programmable platforms, bridging nanotechnology and targeted medicine, as shown in **Figure 4** above.

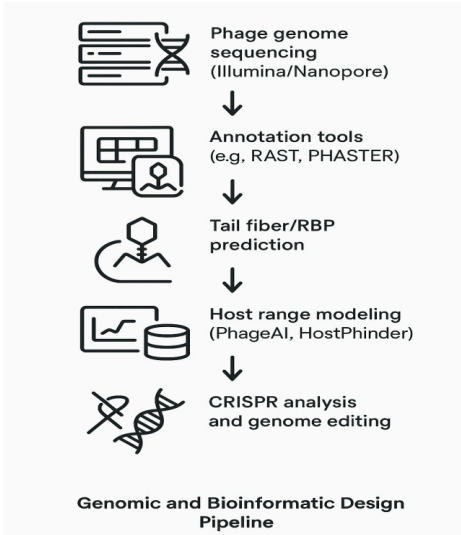


Figure 4. Chemical modification strategies of phage surfaces, including PEGylation, ligand conjugation, and nanoparticle hybridization.

Therapeutic Applications and Challenges

Phages are gaining traction in clinical settings, particularly against multidrug-resistant pathogens.

Infectious Disease

Phage therapy has demonstrated success in treating sepsis, cystic fibrosis lung infections, and non-healing wounds. Case studies support the use of personalized cocktails tailored to the patient’s bacterial isolate [31].

Biofilm Disruption

Phages produce depolymerases and matrix-degrading enzymes that penetrate biofilms, kill metabolically dormant cells, and prevent recolonization. They are being formulated in hydrogels, coatings, and wound dressings [32].

Targeted Drug and Gene Delivery

Engineered phages deliver:

- 1. CRISPR–Cas antimicrobials,
- 2. Antibiotics or peptides,
- 3. Imaging or gene therapy payloads via ligand targeting [33].

Challenges

Barriers include:

- 1. Rapid clearance by immune cells
- 2. Variable tissue distribution
- 3. Lack of standardized regulatory pathways [34].

Solutions under development include PEGylation, susceptibility-matched libraries, and AI-guided phage selection tools.

Phages are positioned to become front-line therapeutics—adaptable, evolvable, and precision-guided.

Genomics, Bioinformatics, and the Future of Precision Phage Therapeutics

Genomic tools have transformed phage discovery and design into a rational, data-driven process.

Genome Sequencing and Annotation

Platforms like RAST, Prokka, and PHASTER rapidly annotate phage genomes, identifying capsid genes, tail fibers, lytic enzymes, and lysogeny-related genes. Phages selected for therapy are strictly lytic to avoid horizontal gene transfer [35]. **Figure 5** expresses **Genomics-Informed Precision Phage Therapy Pipeline**

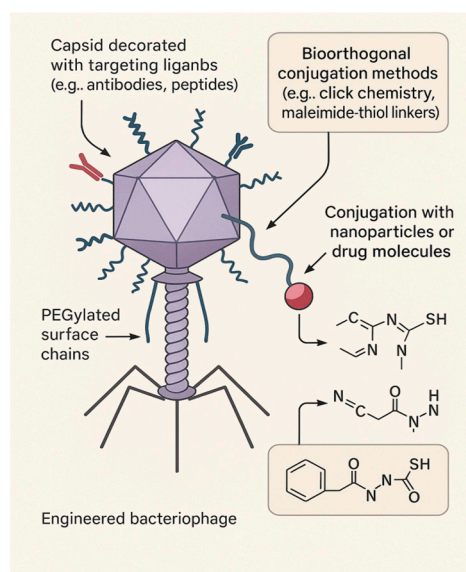


Figure 5. Workflow of personalized phage therapy: pathogen isolation, sequencing, phage matching, and patient-specific formulation.

Comparative Genomics and Host Range Prediction

Tools like PhageAI and HostPhinder predict host range by analyzing tail fiber genes, codon usage, and structural motifs. These methods accelerate candidate phage identification without lengthy wet-lab screening [36].

CRISPR–Phage Co-evolution

Bacterial CRISPR arrays serve as memory banks of prior phage infections. In turn, phages evolve via target mutations or acquire anti-CRISPR (Acr) proteins to evade immunity [37].

Synthetic Genome Engineering

Using lambda red recombineering and CRISPR-based editing, researchers can reprogram phages by deleting lysogeny genes, inserting payloads, or altering host tropism [38].

Toward Personalization

A growing clinical pipeline allows for patient-specific pathogen sequencing, phage matching, and treatment in under a week. This forms the basis for scalable, precision phage therapy [39].

Conclusion and Outlook

Bacteriophages are no longer just biological oddities—they are chemically programmable tools engineered for precision. Their modular architecture, catalytic enzymes, and genetic tractability support the development of targeted, resistance-proof therapies.

This review has demonstrated how modern chemistry, structural biology, and genomics converge to position phages as next-generation antimicrobials.

Recommendations:

1. Prioritize collaboration between chemists, structural biologists, and microbiologists.
2. Frame phage therapy as a modular chemical approach—not a fallback.
3. Build curated libraries of phage structures and enzyme domains.
4. Push for regulatory pathways tailored to customizable biologics.
5. Integrate AI-driven selection and genomic matching into therapy design.

Phages offer a promising solution in the fight against antimicrobial resistance—precise, programmable, and personalized.

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